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WATER SAMPLE ANALYSIS
WITH THE INTEGRATED VIRUS DETECTION SYSTEM

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14. ABSTRACT
This study examined using the Integrated Virus Detection System (IVDS) for rapid monitoring and identification of viruses in various water samples. Initial protocols for determining the presence of viruses in the various waters were explored with the IVDS. The IVDS is a generic virus detector that only requires general reagents. The equipment allows for detection and testing of large volumes and multiple samples of water in a short period of time.

15. SUBJECT TERMS

Virus detection	MS2	Water analysis
Biological agents	Tap water	Chlorine concentration
Electrospray injection	Differential mobility analyzer	
Condensation particle counter	Reverse osmosis water	

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PREFACE

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WATER SAMPLE ANALYSIS
WITH THE INTEGRATED VIRUS DETECTION SYSTEM

1.   INTRODUCTION

This study, using the Integrated Virus Detection System (IVDS), examined the rapid monitoring and identification of viruses in various water samples. Initial protocols for determining the presence of viruses in the various waters were explored with the IVDS, which is a generic virus detector that only requires general reagents. The equipment allows for detection and testing of large volumes and multiple samples of water in a short period of time.

1.1   Water Sample Analysis

The types of water used as starting feed streams in the virus recovery experiments included reverse osmosis (RO), RO with chlorine (RO-Cl), and standard tap water. In addition, several native water samples were collected and analyzed with the IVDS. Examination of several feed streams will determine if either contamination or constituents in the standardized water types will interfere with virus recovery and analysis. The bacteriophage, MS2, was added to the RO, RO-Cl, and tap waters to determine detection and recovery feasibility. MS2 is stable for extended periods of time (months), is not considered pathogenic (BL-1), and is used for numerous viral simulant studies. The IVDS has successfully analyzed MS2 bacteriophage and documented the results in various reports[1, 2, 3].

The IVDS uses electrospray injection, differential mobility analyzer (DMA), and a condensation particle counter (CPC) to analyze viruses. The electrospray module subjects the target liquid sample to a strong electric field, producing a cone that emits a fine jet that breaks up into small droplets. To eliminate the possibility of breakdown (corona discharge) of the air in the plume, as caused by the high electric field, the spray tip is surrounded by a flow of CO_2, which prevents corona discharge. The DMA separates particles by their electrical mobility in air. Particle mobility, which is related to size and charge, will either pass particles through the DMA or the particles will impinge on the walls of the DMA if the mobility does not match the electrical charge. With singly charged particles, which are generated by the electrospray, the mobility becomes a direct measure of the particle size. In the CPC, the sample particles flow in tandem with a saturated working fluid of butanol. The nanosized particles initiate the condensation of the butanol, and the stream is then cooled. A standard optical counter can then count the butanol-condensed particles, and the results are displayed via the supplied software. The IVDS output can be tailored to a variety of PC-based reporting software suites. A more comprehensive look at the IVDS can be found in other U.S. Army Edgewood Chemical Biological Center (ECBC) technical reports [4, 5].

1.2   Chlorinated Water Samples

A sample of MS2 bacteriophage was added to a standard chlorinated water sample, supplied by ECBC, to determine the ability to recover the MS2 and if there is any reduction (fate) of concentration of the virus while in solution. The standard chlorinated water sample had 1.5-2 ppm of free Cl in RO water, as measured the morning the water sample was produced. The sample of MS2 was analyzed before its addition to the chlorinated water.

Figure 1 shows the analysis of the MS2 with its resultant particle count of 493 counts in the 21.67 - 25.9 nm range in the ROI. The IVDS scan of the initial chlorinated water sample in Figure 2 shows no virus or other contamination in the sample. After adding 0.5 mL of the MS2 sample to 1 L of chlorinated water, on the same morning the water was produced, an aliquot was taken and analyzed with the IVDS (Figure 3). Again, there was no contamination present, and the concentration of MS2 in the sample was too low to be detected. The entire liter of spiked water was ultrafiltered to reduce and concentrate the volume from 1 L to 1.2 mL. This ultrafiltration (UF) was accomplished in ~60 min. The remaining volume was analyzed with the IVDS. The reduction/concentration factor of 1 L - 1.2 mL would allow the IVDS to detect the MS2 in the remaining volume. As shown in Figure 4, the MS2 peak shows a count of 3 counts in the 21.67 - 25.9 nm range in the ROI. The large peaks at 16.3 and 17.5 nm are indicative of the protein breakdown products from MS2 that has been disrupted due to chemical means [6, 7].
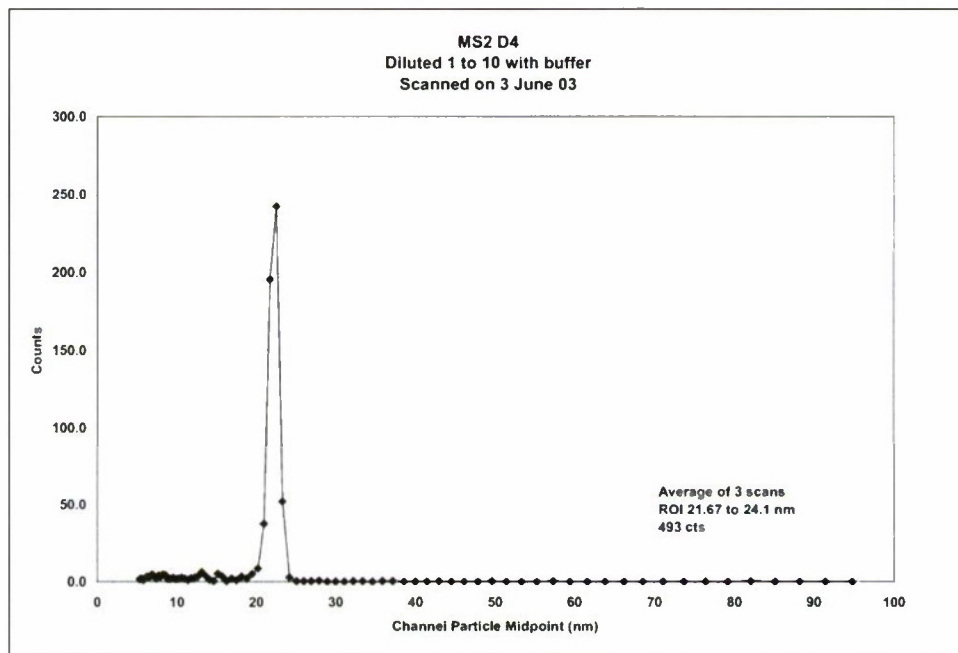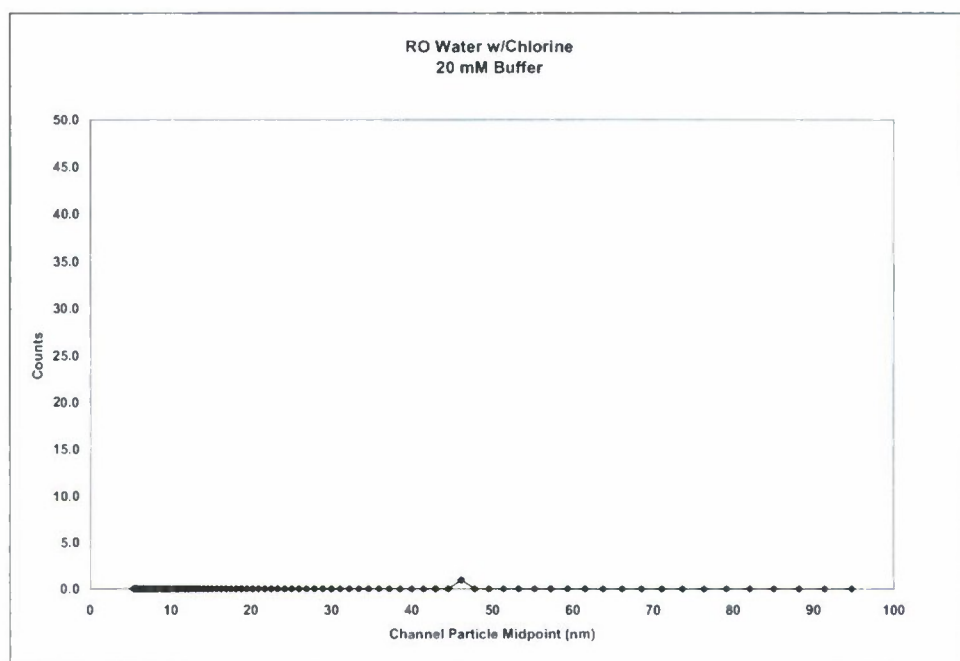

Figure 1. Initial MS2 Sample
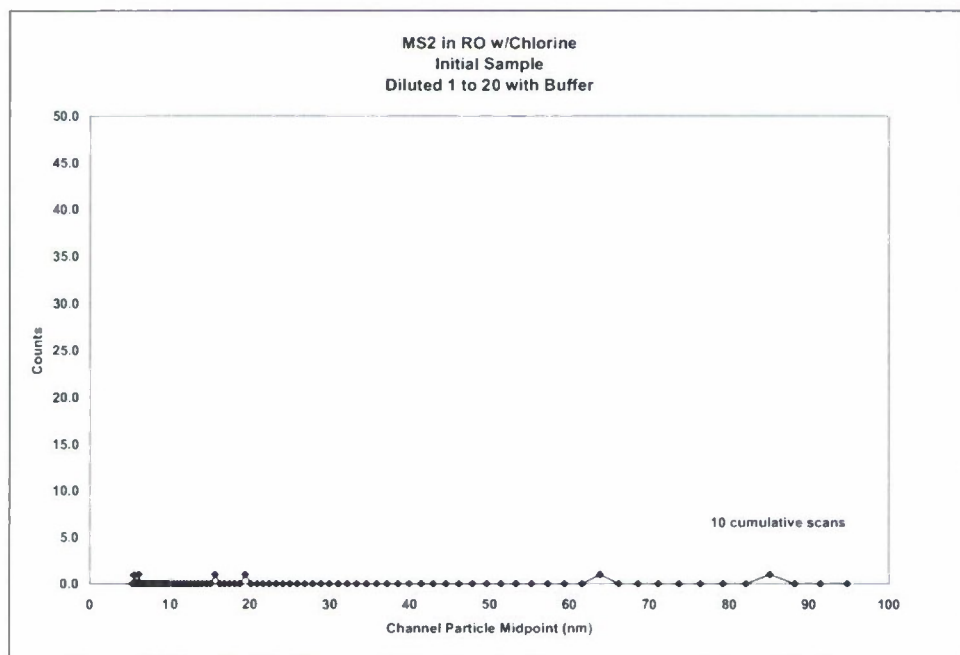
Figure 2. Initial RO Water with Chlorine

Figure 3. Initial RO Water w/Cl after MS2 Addition
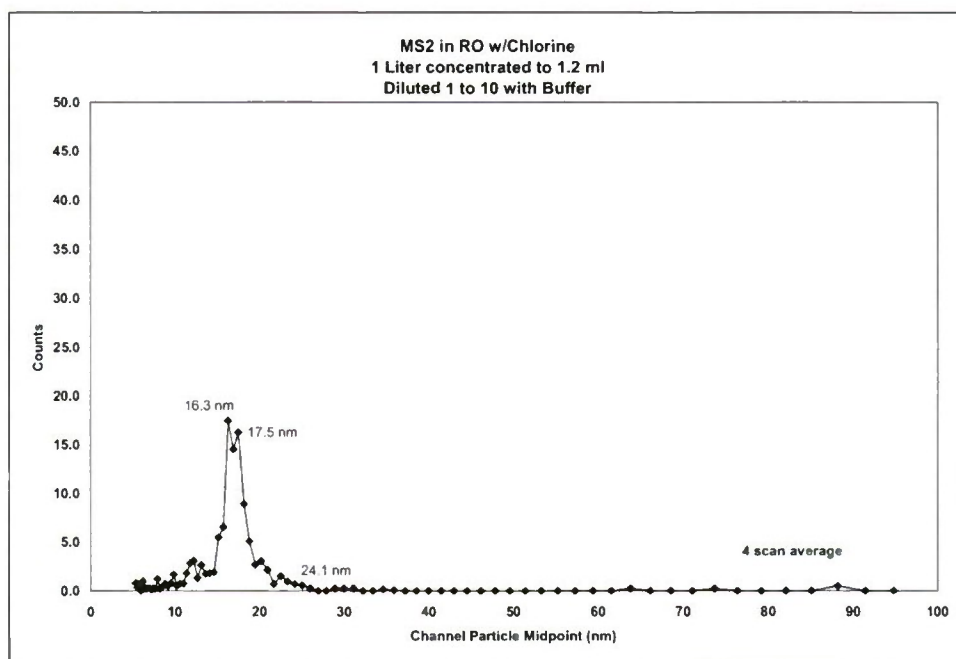
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Figure 4. Water w/Cl and MS2 after Concentration

1.3   Standard Reverse Osmosis Samples

        A sample of MS2 bacteriophage was added to a standard RO water sample, supplied by ECBC, to determine the ability to recover the MS2 from solution. The sample of MS2 was analyzed before addition to the RO water. Figure 5 shows the analysis of the MS2 with a particle count of 814 counts in the 21.67 - 25.9 nm range in the ROI. The IVDS scan of the initial RO water sample in Figure 6 shows no viral or other contamination in the sample. After adding 1.0 mL of the MS2 sample to 2 L of RO water, an aliquot was taken and analyzed with the IVDS (Figure 7). Again, there was no contamination present, and the MS2 was in too low a concentration to be detected. The entire volume of spiked water was ultrafiltered to reduce and concentrate the volume from 2 L to 0.7 mL. The remaining volume was analyzed with the IVDS. The reduction/concentration factor of 2 L to 0.7 mL would allow the IVDS to detect the MS2 in the remaining volume. As shown in Figure 8, the MS2 peak shows a count of 475 in the ROI.
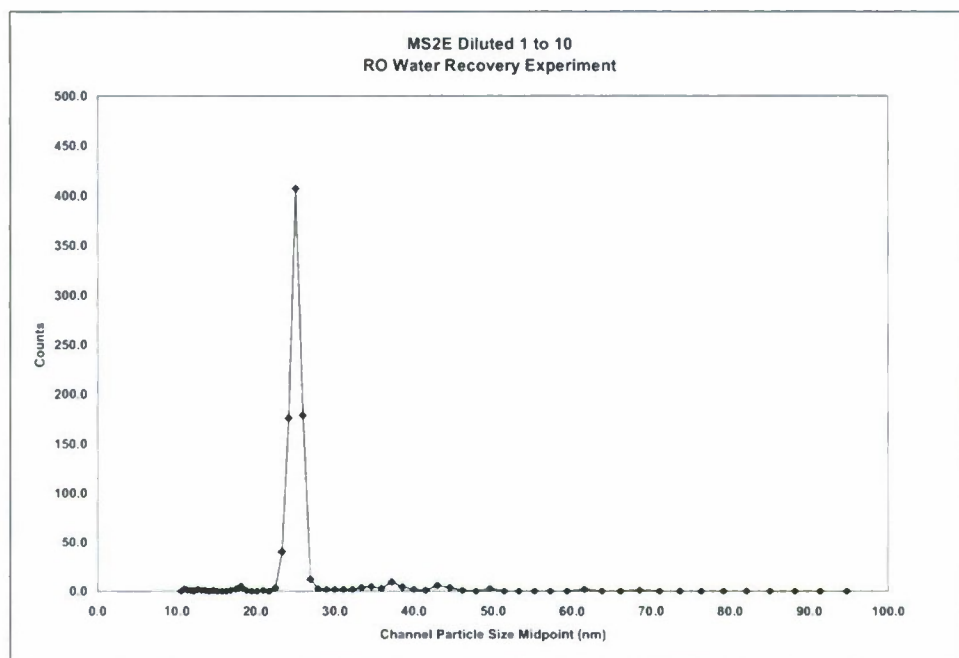
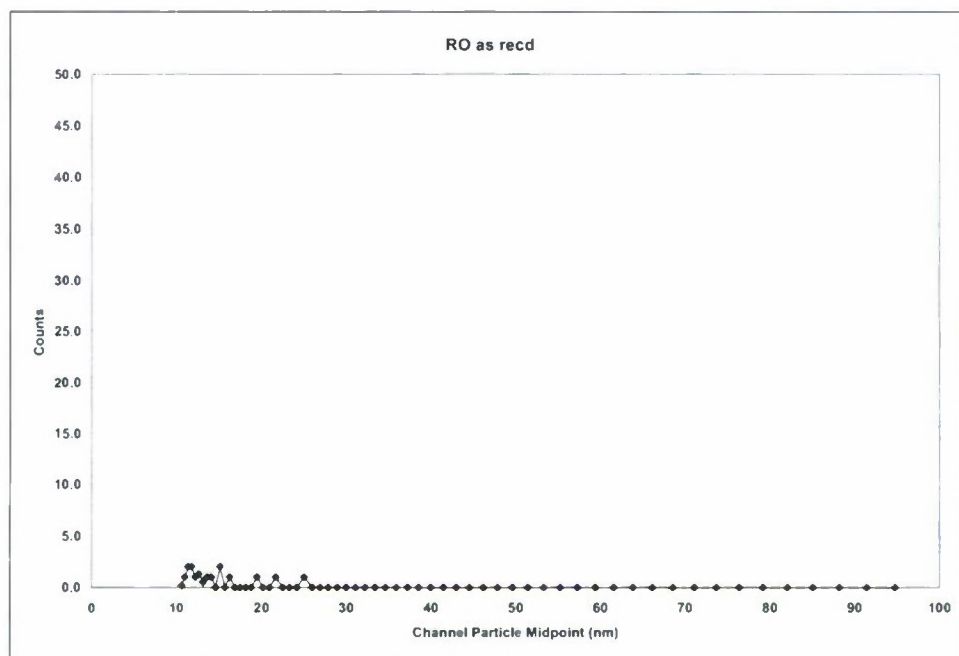Figure 5. Initial MS2 Sample for RO
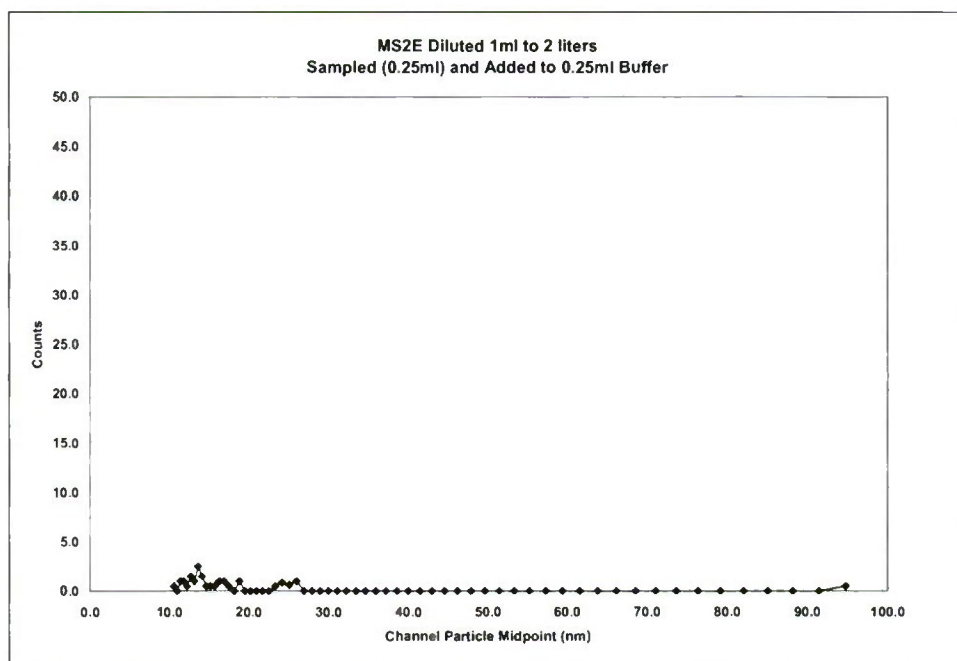

Figure 6. Standard RO Water

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MS2E Diluted 1ml to 2 liters
Sampled (0.25ml) and Added to 0.25ml Buffer

Figure 7. Initial RO Water after MS2 Addition

MS2E Diluted 1 ml to 2 Liters
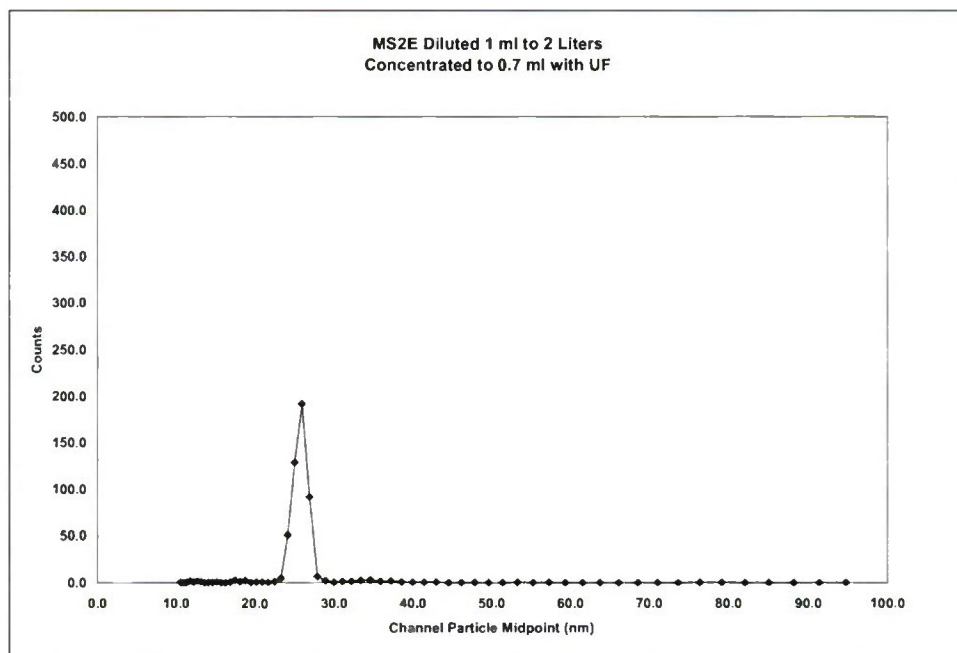**Concentrated to 0.7 ml with UF**

Figure 8. RO Water and MS2 after Concentration

1.4 Standard Tap Water Samples

A sample of MS2 bacteriophage was added to a standard tap water sample, supplied by ECBC, to determine the ability to recover the MS2 and to observe if there was any reduction (fate) while in solution. The standard tap water formula is listed in Table 1. The sample of MS2 added to the tap water was the same sample (Figure 1) used for the chlorinated

water tests. The IVDS scan of the initial tap water sample in Figure 9 shows no viral or other contamination in the sample. After adding 0.5 mL of the MS2 sample to 1 L of tap water, an aliquot was taken and analyzed with the IVDS (Figure 10). Again, there was no contamination present, and the MS2 was in too low a concentration to be detected. The entire liter of spiked water was ultrafiltered to reduce and concentrate the volume from 1 L to 1.0 mL. The remaining volume was analyzed with the IVDS. The reduction/concentration factor of 1 L to 1.0 mL would allow the IVDS to detect the MS2 in the remaining volume. As shown in Figure 11, in the ROI of 21.67 to 25.9 nm, the MS2 peak shows a count of 55. Although the pH of the solution is near neutral (pH=7.65), there are additions of organic material in the tap water, specifically humic and fulvic acids. It is possible that the MS2 bacteriophage has attached to the organic material possibly being removed by filtration, is not in solution, and cannot be counted by the IVDS. Further tests would be required to determine if the organic constituents, or any of the other chemicals, cause the reduction in the quantity of bacteriophage in solution.

Table 1. Constituents of Standard Tap Water

| Salt	Formula	Final Salt Concentration (mg/L)
Sodium bicarbonate	$NaHCO_3$	100.0
Magnesium sulfate	$MgSO_4 \bullet 7H_2O$	13.4
Potassium monophosphate	K_2HPO_4	0.7
Potassium diphosphate	KH_2PO_4	0.3
Ammonium sulfate	$(NH_4)_2SO_4$	0.01
Sodium chloride	$NaCl$	0.01
Ferrous sulfate	$FeSO_4 \bullet 7H_2O$	0.001
Sodium nitrate	$NaNO_3$	1.03
Calcium sulfate	$CaSO_4$	26.9
Organics		Final Concentration (mg/L)
Humic acid		1.01
Fulvic acid		1.01
Physical Parameters		
pH of water matrix = 7.65 @ 29.6 °C		
Conductivity = 175.1 µS		
Stored at 4 °C		
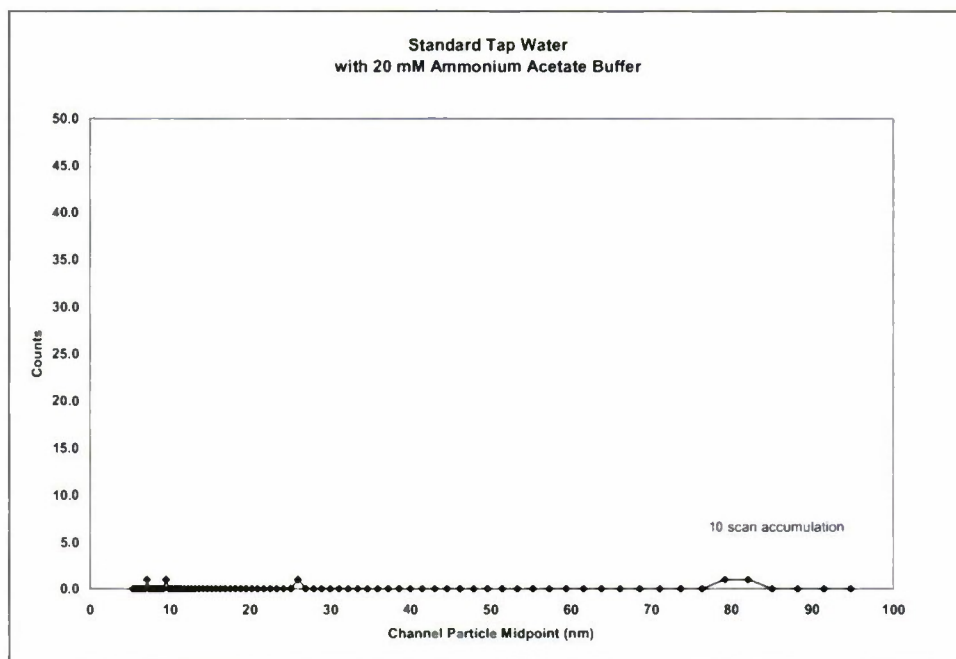
Figure 9. Standard Tap Water
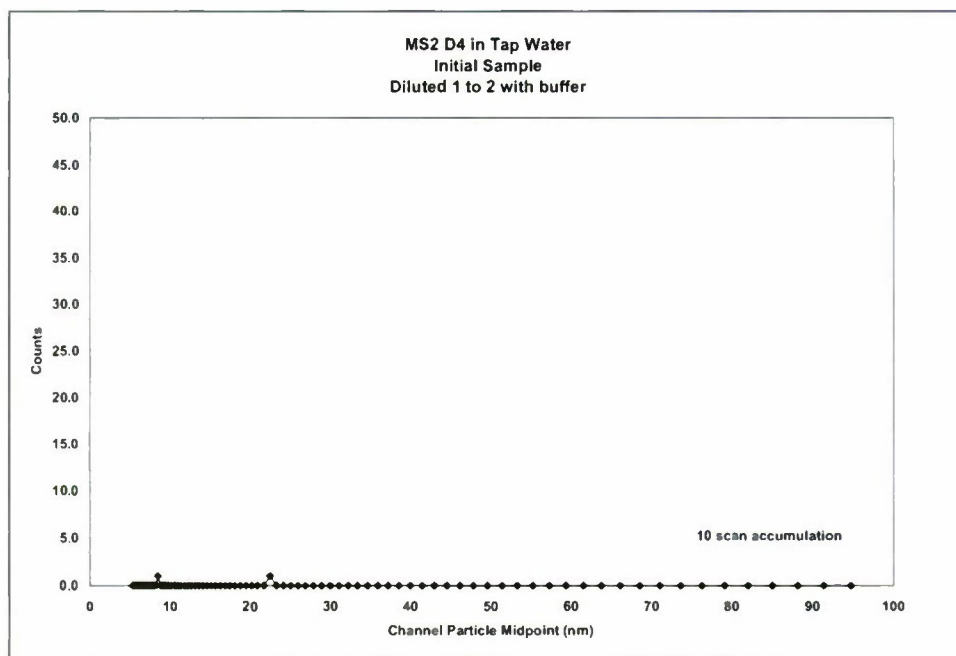
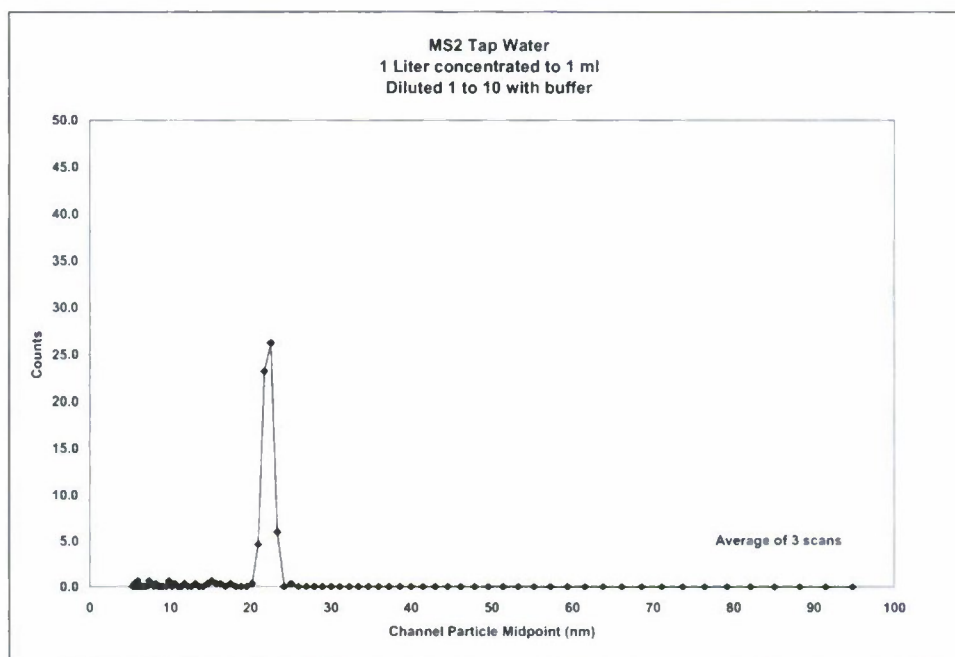Figure 10. Initial Tap Water after MS2 Addition

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Figure 11. RO Tap Water and MS2 after Concentration

1.5 Natural Water Samples

Viruses occur in the natural water environment [8] in quantities that can vary widely with conditions, time of year, environmental variables, etc. Several samples were collected and analyzed with the IVDS. In general, the samples were concentrated with the tangential flow (ultrafiltration) filter system before IVDS analysis. This was done to concentrate the samples to make the analysis more efficient and reduce the need to run long accumulation scans.

Figures 12 to 15 show the IVDS scans from the natural water samples after filtration and concentration. There are small peaks shown that could indicate the presence of viruses in the samples. A larger study with periodic sampling at repeat sites would establish the variations in virus loading in the samples compared to differing environmental variables. Table 2 shows the volume reductions and filters used.
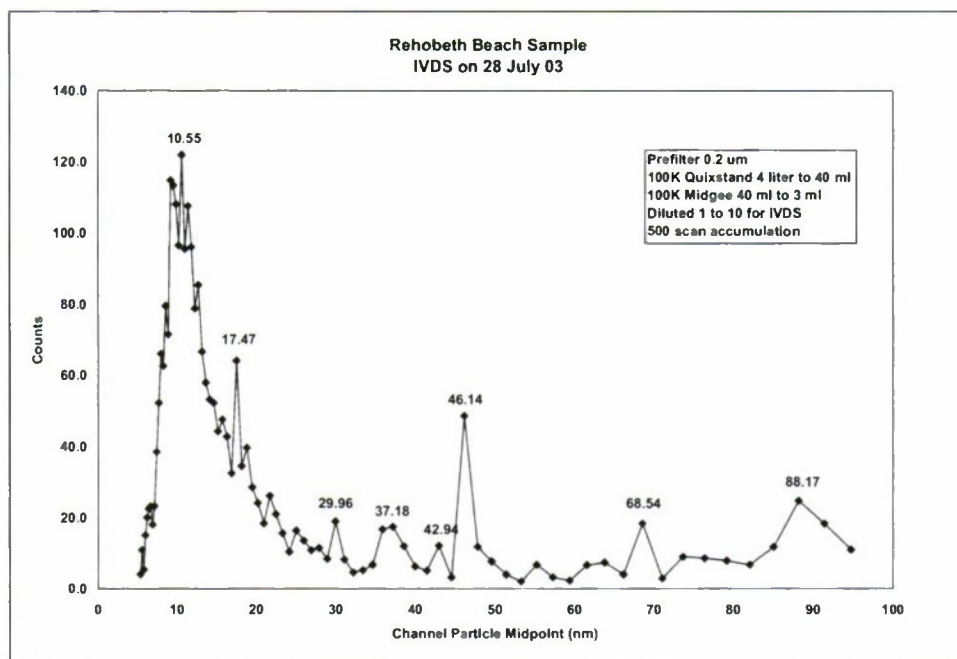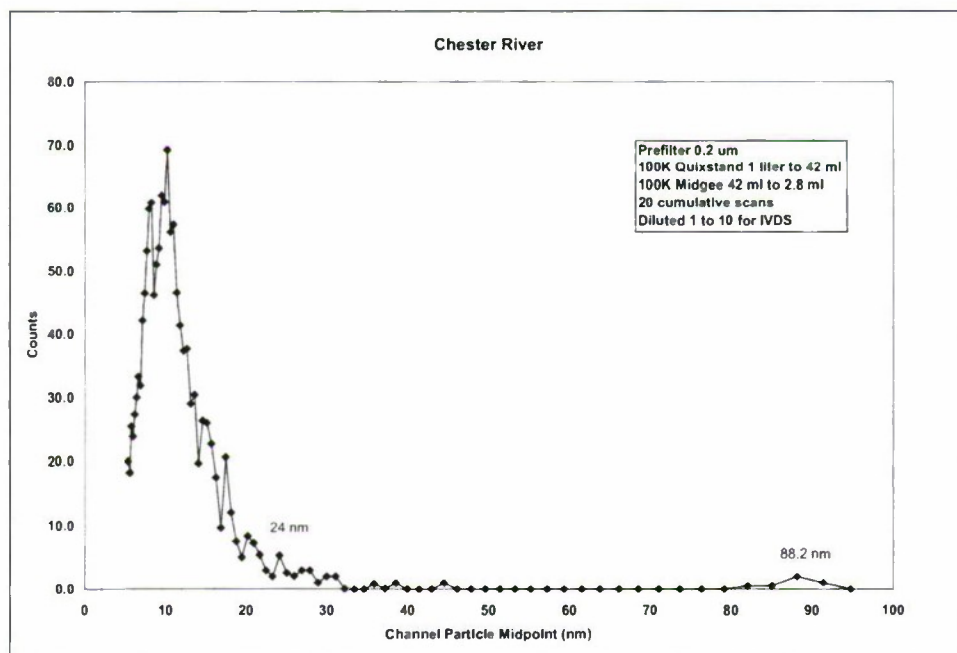
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Figure 12. Rehobeth Beach Virus Analysis

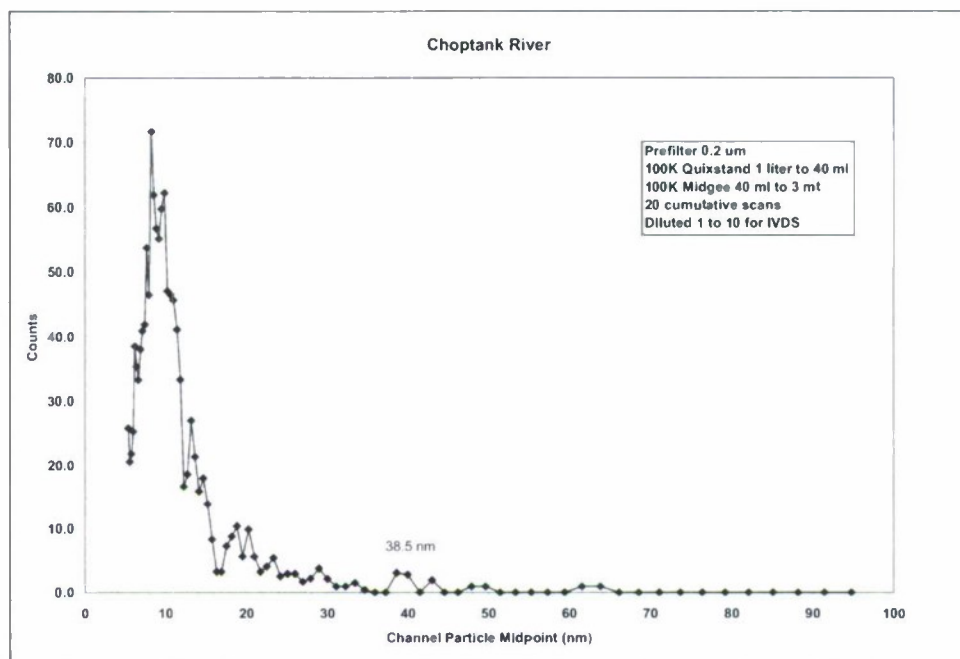Figure 13. Chester River Virus Analysis

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Figure 14. Choptank River Virus Analysis

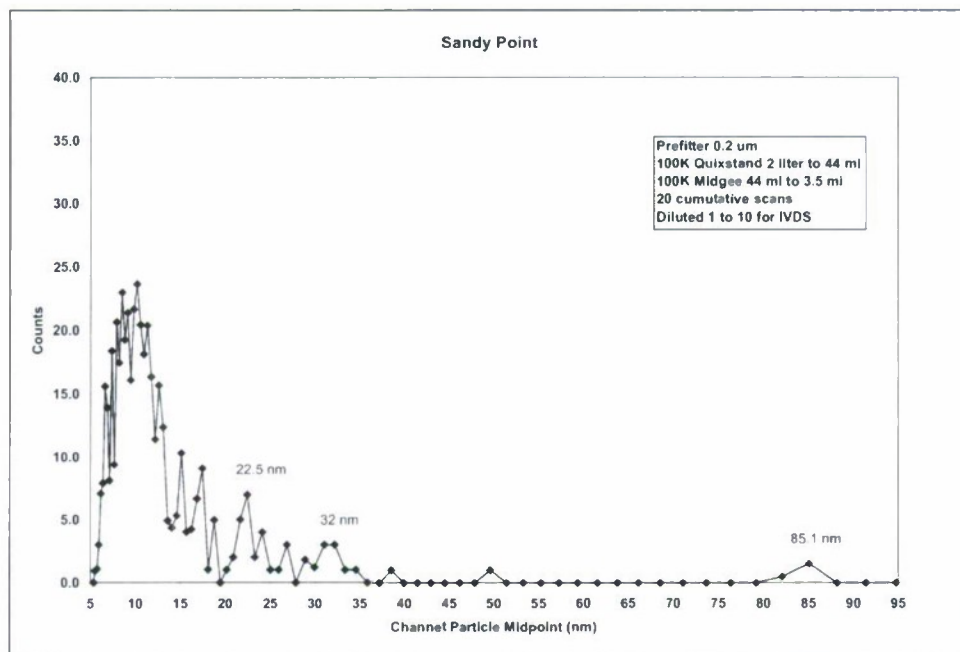Figure 15. Sandy Point Virus Analysis

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Table 2. Filtration Parameters for Natural Water Analysis

Rehobeth Beach			
Filter size	Filter surface area (m^2)	Volume change	Model #
0.2 μm	0.011	4 L - 4 L	3M
100K Da MWCO	0.14	4 L - 42 mL	4X2M
100K Da MWCO	0.0026	40 mL - 3 mL	MM
Chester River			
Filter size	Filter surface area	Volume change	Model #
0.2 μm	0.011	1 L - 1 L	3M
100K Da MWCO	0.014	1 L - 42 mL	3M
100K Da MWCO	0.0026	42 mL - 2.8 mL	MM
Choptank River			
Filter size	Filter surface area	Volume change	Model #
0.2 μm	0.011	1 L - 1 L	3M
100K Da MWCO	0.014	1 L - 40 mL	3M
100K Da MWCO	0.0026	40 mL - 3 mL	MM
Sandy Point			
Filter size	Filter surface area	Volume change	Model #
0.2 μm	0.011	2 L - 2 L	3M
100K Da MWCO	0.14	2 L - 44 mL	4X2M
100K Da MWCO	0.0026	42 mL - 3.5 mL	MM
MWCO – Molecular weight cut-off			

2. DISCUSSION AND CONCLUSIONS

- The Integrated Virus Detection System (IVDS) recovered viruses from most types of water used in this study. The reverse osmosis (RO) and tap water recovery and concentration of viruses was successful. The tap water did seem to reduce some of the MS2 in solution. Further tests with varying concentrations of the constituents of the tap water may determine the effects of the constituents on MS2 recovery. The RO-chlorine (Cl) water seemed to break down the MS2 and leave only virus fragments, which were analyzed after concentration. The Cl containing water needs subsequent analysis to determine the concentrations of Cl that are effective in removing the MS2 from solution. Various concentrations need to be tested for the effects of Cl on MS2, and possibly other virus types could be easily repeated with this type study.

- The IVDS system was able to analyze the large volumes of water in a fairly short period of time. The system reduced the sample volume from 2 L to <2 mL of solution, in <2 h, and completed the analysis. In fact, the actual sampling time for virus analysis is <5 min. The remainder of the time was reducing and concentrating the large volumes for analysis.

- Although the IVDS only shows the size of the virus in question, and does not give a definitive identification, virus types can be classed by their physical size, and preliminary identification of the virus type is possible. Several virus types could be analyzed, if available, in the differing water types. Several viruses could also be analyzed in the same sample to simulate a real world situation. After filtration, the virus analysis takes only a few minutes, and this confirms that the IVDS is a very fast screening tool for initial virus identification.

- The IVDS was able to determine that virus material was probably present in the natural water samples tested. Further tests and possible virus identification of the samples would be possible with a larger program that samples waters periodically and compares the results to environmental variables.

- The ability to resolve several viruses in a single sample with simple processing make the IVDS system useful for monitoring and screening of virus sized particles in water samples of all sorts. In addition, if there are numerous viruses in a sample (e.g., sea water), after a few samples, a baseline can be determined for a given area and followed over time to measure the natural variation in virus sized particles. A change in this natural condition is simple to detect and follow.

- The IVDS is further useful for determining a fate curve for given viruses in water for various conditions such as temperature, pH, and other factors.

- This technique is indicated for detecting viruses and virus like particles in a variety of samples and particularly in water. This technique detects several of these particles in the sample and can indicate the relative concentration of the particles. This is particularly useful when looking for either unknown or unexpected nanometer particles.

- The IVDS performed well with a wide range of water types, including drinking water, environmental fresh water, and seawater.

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